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(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BANDMAN, Olga [US/US]; 366 Anna Avenue, Mountain View, CA 94043 (US). HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Berkeley, CA 94702 (US). TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). KASER, Matthew, R. [GB/US]; 4793 Ewing Road, Castro Valley, CA 94546-1017 (US).

(74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

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           9          18          27          36          45          54
G CCC TCT CCC CGC CCA TAT ATG TAG GGC AGC AGC AGG ATG GGC TTC TGG ACT

          63          72          81          90          99         108
TGG GCG GCC CCT CCG CAG GCG GAC CGG GGG CAA AGG AGG TGG CAT GTC GGT CAG

         117         126         135         144         153         162
GCA CAG CAG GGT CCT GTG TCC GCG CTG AGC CGC GCT CTC CCT GCT CCA GCA AGG

         171         180         189         198         207         216
ACC ATG AGG GCG CTG GAG GGG CCA GGC CTG TCG CTG CTG TGC CTG GTG TTG GCG
  M   R   A   L   E   G   P   G   L   S   L   L   C   L   V   L   A

         225         234         243         252         261         270
CTG CCT GCC CTG CTG CCG GTG CCG GCT GTA CGC GGA GTG GCA GAA ACA CCC ACC
L   P   A   L   L   P   V   P   A   V   R   G   V   A   E   T   P   T

         279         288         297         306         315         324
TAC CCC TGG CGG GAC GCA GAG ACA GGG GAG CGG CTG GTG TGC GCC CAG TGC CCC
Y   P   W   R   D   A   E   T   G   E   R   L   V   C   A   Q   C   P

         333         342         351         360         369         378
CCA GGC ACC TTT GTG CAG CGG CCG TGC CGC CGA GAC AGC CCC ACG ACG TGT GGC
P   G   T   F   V   Q   R   P   C   R   R   D   S   P   T   T   C   G
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(57) Abstract

The invention provides human tumor necrosis factor-R2-like proteins (TR2P) and polynucleotides which identify and encode TR2P. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of TR2P.

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HUMAN TUMOR NECROSIS FACTOR-R2-LIKE PROTEINS

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human tumor necrosis factor-R2-like proteins and to the use of these sequences in the diagnosis, treatment, and prevention of osteogenesis, developmental, reproductive, immunological, and neoplastic disorders.

BACKGROUND OF THE INVENTION

Many eukaryotic cell membranes contain proteins that provide structural support, cell and tissue identity, and autocrine, paracrine and juxtacrine properties for the cell within its environment (McGowan, S.E. (1992) FASEB J. 6:2895-2904). The diverse biochemistry of membrane proteins (MPs) is indicative of the many, often overlapping, roles that are attributed to each distinct molecule. (Grant, D.S. and Kleinman, H.K. (1997) EXS 79:317-333.) While a great number of MPs have been isolated, it remains unclear how the majority of MPs interact with one another or with other molecules residing within the cell membrane. Many MPs have been associated with tissue growth,cell proliferation, tissue or cell differentiation, and cell death. (Taipale, J. and Keski-Oja, J. (1997) FASEB J. 11:51-59; Eleftheriou, C.S. et al. (1991) Mutat. Res. 256:127-138.)

For example, the process of embryonic bone formation involves the creation of an extracellular matrix that mineralizes during the course of tissue maturation. During the life of an individual, this matrix is subject to constant remodeling through the combined actions of osteoblasts (which form mineralized bone) and osteoclasts (which resorb bone). The balance of MP composition, and the resulting bone structure, may be perturbed by biochemical changes that result from congenital, epigenetic, or infectious diseases. (Francomano, C.A. et al. (1996) Curr. Opin. Genet. Dev. 6:301-308.)

MPs also act as important mediators and regulators of the inflammatory response. Leukocytes are primed for inflammatory mediator and cytokine production by ligand-receptor binding during extravasation. (Pakianathan, D.R. (1995) J. Leukoc. Biol. 57:699-702.) Tumor necrosis factor (TNF) is a pleiotropic cytokine that a mediates immune

regulation and inflammatory responses. The cellular responses triggered by TNF are initiated through its interaction with two distinct cell surface receptors, TNF-R1 (55 kDa) and TNF-R2 (75 kDa). (Tartaglia, L.A. and Goeddel, D.V. (1992) Immunol. Today 13:151-153.) Both TNF receptors are part of the larger TNF receptor (TNFR) superfamily whose members, including the Fas antigen, CD27, CD30, and CD40, share characteristic cysteine-rich pseudorepeats in their extracellular domains, the TNFR/NGFR family cysteine-rich region signature. (Smith, C.A. et al. (1994) Cell 76:959-962.) TNF-related cytokines generate partially overlapping cellular responses, including differentiation, proliferation, NF-kB activation, and cell death, by triggering the aggregation of receptor monomers.. (Smith et al. supra.) Although both TNF receptor types are expressed by most cell types, TNF-R1 is responsible for the majority of TNF activities. (Tartaglia and Goeddel, supra.) The contribution of TNF-R2 can be explained in part by the ligand passing model, in which TNF-R2 presents TNF to neighboring TNF-R1 molecules, the latter being signal transducing. (Tartaglia and Goeddel, supra.) Direct TNF-R2 signaling mainly affects lymphoid cells, e.g., proliferation of the CT6 T cell line and induction of granulocyte/macrophage colony-stimulating factor secretion by a T cell hybridoma. (Tartaglia, L.A. et al., (1993) Cell 74:845-853.) In addition, an independent signaling role for TNF-R2 has been demonstrated in TNF-mediated cytotoxicity in some specific cell lines.(Vandenabeele, P. et al. (1995) Trends Cell Biol. 5:392-399.)

The cytoplasmic domains of TNF-R1 and TNF-R2 lack sequence homology, suggesting that they generate distinct activation signals. Beyaert, R. et al. (1995; J. Biol. Chem. 270:23292-23299) identified a TNF-R kinase as casein kinase 1 (CK-1) and observed that phosphorylation by CK-1 negatively regulates TNF-R2-mediated signaling to TNF-induced apoptosis. Inappropriate TNF expression has been linked to the development of diseases such as septic shock and certain autoimmune disorders. (Beyaert, R. et al. supra.) Osteoprotegerin is a recently identified member of the TNF-R2 family which regulates bone resorption. Osteoprotegerin blocks differentiation of precursor cells to osteoclasts and blocks ovariectomy-associated bone loss in rats. (Simonet, W.S. et al. (1997) Cell 89:309-319.)

The discovery of new human tumor necrosis factor-R2-like proteins and the polynucleotides encoding these human tumor necrosis factor-R2-like proteins satisfies a need in the art by providing new compositions which are useful in the diagnosis,

treatment, and prevention of osteogenesis, developmental, reproductive, immunological, and neoplastic disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, human tumor necrosis factor-R2-like proteins, referred to collectively as "TR2P" and individually as "TR2P-1" and "TR2P-2." In one aspect, the invention provides a substantially purified polypeptide, TR2P, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3.

The invention further provides a substantially purified variant of TR2P having at least 90% amino acid identity to the amino acid sequences of SEQ ID NO:1 or SEQ ID NO:3, or to a fragment of either of these sequences. The invention also provides an isolated and purified polynucleotide sequence encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3.

Additionally, the invention provides a composition comprising a polynucleotide sequence encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3. The invention further provides an isolated and purified polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3, as well as an isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3.

The invention also provides an isolated and purified polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID

NO:2, SEQ ID NO:4, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:4. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:4, as well as an isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:4.

The invention further provides an expression vector containing at least a fragment of the polynucleotide sequence encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, or a fragment of SEQ ID NO:3, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide sequence encoding TR2P under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified TR2P having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, or a fragment of SEQ ID NO:3 in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, or a fragment of SEQ ID NO:3, as well as a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing an osteogenesis disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified TR2P.

The invention also provides a method for treating or preventing a developmental

disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified TR2P.

The invention also provides a method for treating or preventing a reproductive disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified TR2P.

The invention also provides a method for treating or preventing an immunological disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of TR2P.

The invention also provides a method for treating or preventing a neoplastic disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of TR2P.

The invention also provides a method for detecting a polynucleotide encoding TR2P in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence encoding the polypeptide comprising SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, or a fragment of SEQ ID NO:3 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding TR2P in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, and 1C show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of TR2P-1. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

Figures 2A, 2B, 2C, and 2D show the amino acid sequence (SEQ ID NO:3) and nucleic acid sequence (SEQ ID NO:4) of TR2P-2. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

Figures 3A, 3B, and 3C show the amino acid sequence alignments among TR2P-1 (1542861; SEQ ID NO:1), human osteoprotegrin (GI 2072185; SEQ ID NO:5), and human

TNF-R2 (GI 1469541; SEQ ID NO:6), produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 4A, 4B, and 4C show the amino acid sequence alignments among TR2P-2 (2581223; SEQ ID NO:3), human TNF-R2 (truncated sequence, V-83 to S-461: GI 1469541; SEQ ID NO:6), and human osteoprotegrin (truncated sequence, L-90 to L-401: GI 2072185; SEQ ID NO:5), produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"TR2P," as used herein, refers to the amino acid sequences of substantially purified

TR2P obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist," as used herein, refers to a molecule which, when bound to TR2P, increases or prolongs the duration of the effect of TR2P. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of TR2P.

An "allele" or an "allelic sequence," as these terms are used herein, is an alternative form of the gene encoding TR2P. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding TR2P, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same TR2P or a polypeptide with at least one functional characteristic of TR2P. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding TR2P, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding TR2P. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent TR2P. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of TR2P is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments", "immunogenic fragments", or "antigenic fragments" refer to fragments of TR2P which are preferably about 5 to about 15 amino acids in length and which retain some biological activity or immunological activity of TR2P. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, pp.1-5, Cold Spring Harbor Press, Plainview, NY.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to TR2P, decreases the amount or the duration of the effect of the biological or immunological activity of TR2P. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of TR2P.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fa, $F(ab')_2$, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind TR2P polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic

determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to a specific nucleic acid sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic TR2P, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotide sequences encoding TR2P or fragments of TR2P may be employed as hybridization probes. The probes may be stored

in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

The phrase "consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence .

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding TR2P, by northern analysis is indicative of the presence of nucleic acids encoding TR2P in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding TR2P.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative," as used herein, refers to the chemical modification of TR2P, of a polynucleotide sequence encoding TR2P, or of a polynucleotide sequence complementary to a polynucleotide sequence encoding TR2P. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains a at least one biological or immunological function of the polypeptide from which it was derived.

The term "homology," as used herein, refers to a degree of complementarity. There may be partial homology or complete homology. The word "identity" may substitute for the word "homology." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred

to as "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially homologous sequence or hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% homology or identity). In the absence of non-specific binding, the substantially homologous sequence or probe will not hybridize to the second non-complementary target sequence.

"Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 10 Kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term "hybridization complex" as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or

nucleotides, respectively, to the sequence found in the naturally occurring molecule.

The term "microarray," as used herein, refers to an array of distinct polynucleotides or oligonucleotides arrayed on a substrate, such as paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The term "modulate," as it appears herein, refers to a change in the activity of TR2P. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of TR2P.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to an oligonucleotide, nucleotide, polynucleotide, or any fragment thereof, to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which are greater than about 60 nucleotides in length, and most preferably are at least about 100 nucleotides, at least about 1000 nucleotides, or at least about 10,000 nucleotides in length.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimers,""primers," "oligomers," and "probes," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA and RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding TR2P, or fragments thereof, or TR2P itself may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or

membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA (in solution or bound to a solid support); a tissue; a tissue print; and the like.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein recognized by the binding molecule (i.e., the antigenic determinant or epitope). For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit hybridization between polynucleotide sequences and the claimed polynucleotide sequences. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In particular, hybridization could occur under high stringency conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 μg/ml sheared and denatured salmon sperm DNA. Hybridization could occur under reduced stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Variations on the above ranges and conditions are well known in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, and refers to cells which transiently express the inserted DNA or RNA for limited periods of time.

A "variant" of TR2P, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

THE INVENTION

The invention is based on the discovery of new human tumor necrosis factor-R2-like proteins (TR2P), the polynucleotides encoding TR2P, and the use of these compositions for the diagnosis, treatment, or prevention of osteogenesis, developmental, reproductive, immunological, and neoplastic disorders.

Nucleic acids encoding the TR2P-1 of the present invention were first identified in Incyte Clone 1533650 from the spleen cDNA library (SPLNNOT04) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 1533650 and 1559031 (SPLNNOT04), 3219886 (COLNNON03), 1339238 (COLNTUT03), 1542861 (PROSTUT04), 3257322 (OVARTUN01), and 2613221

(SINIUCT01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. TR2P-1 is 245 amino acids in length and has a potential N-glycosylation site at N-173, a potential protein kinase A or G phosphorylation site at residue S-66, two potential casein kinase II phosphorylation sites at residues S_{163} and S_{187}, two potential protein kinase C phosphorylation sites at residues S_{202} and S_{225}, one potential tyrosine kinase phosphorylation site at residue Y_{36}, one TNFR/NGFR family cysteine-rich region signature between residues C_{77} and C_{113}, and two phospholipase A_2 active site signatures between residues C_{126} and C_{132}, and C_{168} and C_{174}. As shown in Figures 3A, 3B, and 3C, TR2P-1 has chemical and structural homology with human osteoprotegrin (GI 2072185: SEQ ID NO:5) and human TNF-R2 (GI 1469541; SEQ ID NO:6). In particular, TR2P-1 and human osteoprotegrin and human TNF-R2 share 31% and 27% identity, respectively. In addition, TR2P-1 and human osteoprotegrin share one potential N-glycosylation site, one potential protein kinase C phosphorylation site, one TNFR/NGFR family cysteine-rich region signature, and two phospholipase A_2 active site signatures. In addition, TR2P-1 and human TNF-R2 share one potential casein kinase II phosphorylation site, one potential protein kinase C phosphorylation site, one TNFR/NGFR family cysteine-rich region signature, one phospholipase A_2 active site signature, and have similar isoelectric points, 6.6 and 5.9, respectively. Northern analysis shows the expression of this sequence in various libraries, at least 58% of which are immortalized or cancerous and at least 41% of which involve immune response. Of particular note is the expression of TR2P-1 in gastrointestinal, reproductive, hematopoietic, cardiovascular, connective, and fetal tissues.

Nucleic acids encoding the TR2P-2 of the present invention were first identified in Incyte Clone 2581223 from the kidney cDNA library (KIDNTUT13) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:4, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2581223 (KIDNTUT13), 260827 (HNT2RAT01), 2482038 (SMCANOT01), and 2623152 (KERANOT02).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:3. TR2P-2 is 393 amino acids in length and has six potential N-glycosylation sites at residues N_{24}, N_{135}, N_{140}, N_{161}, N_{172}, and N_{322}, two potential

protein kinase A or G phosphorylation sites at residues T_{44} and S_{257}, eight potential casein kinase II phosphorylation sites at residues T_{10}, T_{44}, T_{58}, S_{152}, T_{157}, T_{202}, S_{324}, and T_{385}, nine potential protein kinase C phosphorylation sites at residues T_{10}, S_{144}, S_{164}, S_{252}, S_{256}, T_{259}, S_{324}, T_{350}, and T_{389}, and one phospholipase A_2 active site signature between residues C_{-27} and C_{-33}. As shown in Figures 4A, 4B, and 4C, TR2P-2 has chemical and structural homology with human TNF-R2 (truncated sequence, V_{83} to S_{461}: GI 1469541; SEQ ID NO:6) and human osteoprotegrin (truncated sequence, L_{90} to L_{401}: GI 2072185: SEQ ID NO:5). In particular, TR2P-2 and human TNF-R2 and human osteoprotegrin share 17% and 18% identity, respectively. In addition, TR2P-2 and human TNF-R2 share two potential protein kinase A or G phosphorylation sites, four potential casein kinase II phosphorylation sites, two potential protein kinase C phosphorylation sites. In addition, TR2P-2 and human osteoprotegrin share two potential protein kinase C phosphorylation sites, one phospholipase A_2 active site signature, and have similar isoelectric points, 8.8 and 9.2, respectively. Northern analysis shows the expression of this sequence in various libraries, at least 93% of which are immortalized or cancerous and at least 29% of which involve immune response. Of particular note is the expression of TR2P-2 in dermal, gastrointestinal, hematopoietic, reproductive, nervous, urologic, and cardiovascular tissues.

The invention also encompasses TR2P variants. A preferred TR2P variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the TR2P amino acid sequence, and which contains at least one functional or structural characteristic of TR2P.

The invention also encompasses polynucleotides which encode TR2P. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:2, which encodes a TR2P, as shown in Figures 1A, 1B, and 1C. In a further embodiment, the invention encompasses the polynucleotide sequence comprising the sequence of SEQ ID NO:4, as shown in Figures 2A, 2B, 2C, and 2D, which encodes a TR2P.

The invention also encompasses a variant of a polynucleotide sequence encoding TR2P. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding TR2P. A particular aspect of

the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:2. The invention further encompasses a polynucleotide variant of SEQ ID NO:4 having at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:4. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of TR2P.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding TR2P, some bearing minimal homology to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring TR2P, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode TR2P and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring TR2P under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding TR2P or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding TR2P and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode TR2P and TR2P derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding TR2P or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:2, SEQ ID NO:4, a fragment of SEQ ID NO:2, or a fragment of SEQ ID NO:4, under various conditions of stringency as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511.)

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp., Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE Amplification System marketed by GIBCO/BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

The nucleic acid sequences encoding TR2P may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus. (Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) In particular, genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region. (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) The primers may be designed using commercially available software such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to

72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR. Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable in that they will include more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., Genotyper™ and Sequence Navigator™, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode TR2P may be used in recombinant DNA molecules to direct expression of TR2P, or fragments or functional equivalents thereof, in appropriate host

cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced, and these sequences may be used to clone and express TR2P.

As will be understood by those of skill in the art, it may be advantageous to produce TR2P-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter TR2P encoding sequences for a variety of reasons including, but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding TR2P may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of TR2P activity, it may be useful to encode a chimeric TR2P protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the TR2P encoding sequence and the heterologous protein sequence, so that TR2P may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding TR2P may be synthesized, in whole or in part, using chemical methods well known in the art. (Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of TR2P, or a fragment thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography. (Chiez, R.M. and Regnier, F.Z. (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (the Edman degradation procedure described in Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY.) Additionally, the amino acid sequence of TR2P, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active TR2P, the nucleotide sequences encoding TR2P or derivatives thereof may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding TR2P and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989; Molecular Cloning, A Laboratory Manual, ch. 4, 8, and 16-17, Cold Spring Harbor Press, Plainview, NY) and Ausubel, F.M. et al. (1995 and periodic supplements; Current Protocols in Molecular Biology, ch. 9, 13, and 16, John Wiley & Sons, New York, NY.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding TR2P. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The invention is not limited by the host cell employed.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector (i.e., enhancers, promoters, and 5' and 3' untranslated regions) which interact with host cellular proteins to carry out transcription and translation. Such elements may

vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, La Jolla, CA) or pSport1™ plasmid (GIBCO/BRL), and the like, may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding TR2P, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for TR2P. For example, when large quantities of TR2P are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding TR2P may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced, pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509), and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used. For reviews, see Ausubel (supra) and Grant et al. (1987; Methods Enzymol. 153:516-544).

In cases where plant expression vectors are used, the expression of sequences encoding TR2P may be driven by any of a number of promoters. For example, viral

promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews. (See, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

An insect system may also be used to express TR2P. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding TR2P may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of TR2P will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which TR2P may be expressed. (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227.)

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding TR2P may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing TR2P in infected host cells. (Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of about 6 Mb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of

sequences encoding TR2P. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding TR2P and its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding-sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular cell system used, such as those described in the literature. (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding, and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long term, high yield production of recombinant proteins, stable expression is preferred. For example, cell lines capable of stably expressing TR2P can be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase genes (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase genes (Lowy, I. et al. (1980) Cell 22:817-23), which can be employed in *tk*⁻ or *apr*⁻ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); *npt* confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14); and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, *TR2PB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine. (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51.) Recently, the use of visible markers has gained popularity with such markers as anthocyanins, ß glucuronidase and its substrate GUS, and luciferase and its substrate luciferin. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding TR2P is inserted within a marker gene sequence, transformed cells containing sequences encoding TR2P can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding TR2P under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding TR2P and express TR2P may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

The presence of polynucleotide sequences encoding TR2P can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or

fragments of polynucleotides encoding TR2P. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding TR2P to detect transformants containing DNA or RNA encoding TR2P.

A variety of protocols for detecting and measuring the expression of TR2P, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on TR2P is preferred, but a competitive binding assay may be employed. These and other assays are well described in the art, for example, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, Section IV, APS Press, St Paul, MN) and in Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding TR2P include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding TR2P, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, MI), Promega (Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding TR2P may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode TR2P may be designed to contain signal sequences which direct secretion of TR2P through a prokaryotic

or eukaryotic cell membrane. Other constructions may be used to join sequences encoding TR2P to nucleotide sequences encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences, such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA), between the purification domain and the TR2P encoding sequence may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing TR2P and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on immobilized metal ion affinity chromatography (IMAC; described in Porath, J. et al. (1992) Prot. Exp. Purif. 3: 263-281)), while the enterokinase cleavage site provides a means for purifying TR2P from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

Fragments of TR2P may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of TR2P may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural homology exists between TR2P-1 and human osteoprotegrin (GI 2072185) and human TNF-R2 (GI 1469541). In addition, TR2P-1 is expressed in tumor, immunological, gastrointestinal, reproductive, hematopoietic, cardiovascular, connective, and fetal tissues. Therefore, TR2P-1 appears to play a role in osteogenesis, developmental, reproductive, immunological, and neoplastic disorders.

Chemical and structural homology exists between TR2P-2 and human TNF-R2 (GI 1469541) and human osteoprotegrin (GI 2072185). In addition, TR2P-2 is expressed in tumor, immunological, dermal, gastrointestinal, hematopoietic, reproductive, nervous,

urologic, and cardiovascular tissues. Therefore, TR2P-2 appears to play a role in reproductive, immunological, and neoplastic disorders.

Therefore, in one embodiment, TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent an osteogenesis disorder. Such disorders can include, but are not limited to, achondroplasia, Caffey disease, craniometaphyseal dysplasia, osteopetrosis, osteoporosis-pseudoglioma syndrome, Paget disease of bone, parastremmatic dwarfism, and polyostotic osteolytic dysplasia.

In another embodiment, a vector capable of expressing TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent an osteogenesis disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified TR2P in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent an osteogenesis disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of TR2P may be administered to a subject to treat or prevent an osteogenesis disorder including, but not limited to, those listed above.

In one embodiment, TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent a developmental disorder. The term "developmental disorder" refers to any disorder associated with development or function of a tissue, organ, or system of a subject (such as the brain, adrenal gland, kidney, skeletal or reproductive system). Such disorders can include, but are not limited to, renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Syndenham's chorea and cerebral palsy, spinal bifida, and congenital glaucoma, cataract, or sensorineural hearing loss.

In another embodiment, a vector capable of expressing TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially

purified TR2P in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of TR2P may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those listed above.

In one embodiment, TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder. Such disorders can include, but are not limited to, disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, autoimmune disorders, ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, and prostatitis, carcinoma of the male breast and gynecomastia.

In another embodiment, a vector capable of expressing TR2P or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified TR2P in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of TR2P may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those listed above.

In a further embodiment, an antagonist of TR2P may be administered to a subject to treat or prevent an immunological disorder. Such a disorder may include, but is not limited to, AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia,

irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, Werner syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma. In one aspect, an antibody which specifically binds TR2P may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express TR2P.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding TR2P may be administered to a subject to treat or prevent an immunological disorder including, but not limited to, those described above.

In a further embodiment, an antagonist of TR2P may be administered to a subject to treat or prevent a neoplastic disorder. Such a disorder may include, but is not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one aspect, an antibody which specifically binds TR2P may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express TR2P.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding TR2P may be administered to a subject to treat or prevent a neoplastic disorder including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of TR2P may be produced using methods which are generally

known in the art. In particular, purified TR2P may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind TR2P. Antibodies to TR2P may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with TR2P or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to TR2P have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of TR2P amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to TR2P may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule

with appropriate antigen specificity and biological activity, can be used. (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce TR2P-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-11123.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837, and Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for TR2P may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (Huse, W.D. et al. (1989) Science 254:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between TR2P and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering TR2P epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

In another embodiment of the invention, the polynucleotides encoding TR2P, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding TR2P may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding TR2P. Thus, complementary molecules or fragments may be used to modulate TR2P activity, or to

achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding TR2P.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequence complementary to the polynucleotides of the gene encoding TR2P. These techniques are described, for example, in Sambrook (supra) and in Ausubel (supra.)

Genes encoding TR2P can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof encoding TR2P. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding TR2P. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, pp. 163-177, Futura Publishing Co., Mt. Kisco, NY.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by

endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding TR2P.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding TR2P. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or

by polycationic amino polymers may be achieved using methods which are well known in the art, such as those described in Goldman, C.K. et al. (1997; Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of TR2P, antibodies to TR2P, and mimetics, agonists, antagonists, or inhibitors of TR2P. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules

(optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of TR2P, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays of neoplastic cells, for example, or in animal models, usually mice, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example TR2P or fragments thereof, antibodies of TR2P, and agonists, antagonists or inhibitors of TR2P, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED50 (the dose therapeutically

effective in 50% of the population) or LD50 (the dose lethal to 50% of the population) statistics. The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the LD50/ED50 ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind TR2P may be used for the diagnosis of disorders characterized by expression of TR2P, or in assays to monitor patients being treated with TR2P or agonists, antagonists, and inhibitors of TR2P. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for TR2P include methods which utilize the antibody and a label to detect TR2P in human body fluids or in extracts of cells

or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent joining with a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring TR2P, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of TR2P expression. Normal or standard values for TR2P expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to TR2P under conditions suitable for complex formation The amount of standard complex formation may be quantified by various methods, preferably by photometric means. Quantities of TR2P expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding TR2P may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of TR2P may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of TR2P, and to monitor regulation of TR2P levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding TR2P or closely related molecules may be used to identify nucleic acid sequences which encode TR2P. The specificity of the probe, whether it is made from a highly specific region (e.g., the 5' regulatory region) or from a less specific region (e.g., the 3' coding region), and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding TR2P, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the TR2P encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequences of SEQ ID NO:2, SEQ ID NO:4, or from genomic sequences including promoter and enhancer elements and introns of the naturally

occurring TR2P.

Means for producing specific hybridization probes for DNAs encoding TR2P include the cloning of polynucleotide sequences encoding TR2P or TR2P derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding TR2P may be used for the diagnosis of a disorder associated with expression of TR2P. Examples of such a disorder include, but are not limited to, an osteogenesis disorder such as achondroplasia, Caffey disease, craniometaphyseal dysplasia, osteopetrosis, osteoporosis-pseudoglioma syndrome, Paget disease of bone, parastremmatic dwarfism, and polyostotic osteolytic dysplasia; a developmental disorder such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Syndenham's chorea and cerebral palsy, spinal bifida, and congenital glaucoma, cataract, or sensorineural hearing loss; a reproductive disorder such as disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, autoimmune disorders, ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, and prostatitis, carcinoma of the male breast and gynecomastia; an immunological disorder such as AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel

syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, Werner syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, and extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma; and a neoplastic disorder such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding TR2P may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patient biopsies to detect altered TR2P expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding TR2P may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding TR2P may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding TR2P in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of TR2P, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding TR2P, under conditions suitable for hybridization or amplification. Standard hybridization may be

quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding TR2P may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding TR2P, or a fragment of a polynucleotide complementary to the polynucleotide encoding TR2P, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of TR2P include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244, and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of

the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to produce a transcript image) and to identify genetic variants, mutations, and polymorphisms. This information may be used in determining gene function, in understanding the genetic basis of a disorder, in diagnosing a disorder, and in developing and monitoring the activities of therapeutic agents.

In one embodiment, the microarray is prepared and used according to methods known in the art, such as those described in published PCT application WO95/11995 (Chee et al.), Lockhart, D. J. et al. (1996; Nat. Biotech. 14:1675-1680), and Schena, M. et al. (1996; Proc. Natl. Acad. Sci. 93:10614-10619.)

The microarray is preferably composed of a large number of unique single-stranded nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6 to 60 nucleotides in length, more preferably about 15 to 30 nucleotides in length, and most preferably about 20 to 25 nucleotides in length. For a certain type of microarray, it may be preferable to use oligonucleotides which are about 7 to 10 nucleotides in length. The microarray may contain oligonucleotides which cover the known 5' or 3' sequence, or may contain sequential oligonucleotides which cover the full length sequence or unique oligonucleotides selected from particular areas along the length of the sequence. Polynucleotides used in the microarray may be oligonucleotides specific to a gene or genes of interest in which at least a fragment of the sequence is known or oligonucleotides specific to one or more unidentified cDNAs common to a particular cell or tissue type or to a normal, developmental, or disease state. In certain situations, it may be appropriate to use pairs of oligonucleotides on a microarray. The pairs will be identical, except for one nucleotide preferably located in the center of the sequence. The second oligonucleotide in the pair (mismatched by one) serves as a control. The number of oligonucleotide pairs may range from about 2 to 1,000,000.

In order to produce oligonucleotides to a known sequence for a microarray, the gene of interest is examined using a computer algorithm which starts at the 5' end, or, more preferably, at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary structure that may interfere with

hybridization. In one aspect, the oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon, any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

In one aspect, the oligonucleotides may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, such as that described in published PCT application WO95/251116 (Baldeschweiler et al.). In another aspect, a grid array analogous to a dot or slot blot (HYBRIDOT® apparatus, GIBCO/BRL) may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system or thermal, UV, mechanical or chemical bonding procedures. In yet another aspect, an array may be produced by hand or by using available devices, materials, and machines (including Brinkmann® multichannel pipettors or robotic instruments), and may contain 8, 24, 96, 384, 1536, or 6144 oligonucleotides, or any other multiple from 2 to 1,000,000 which lends itself to the efficient use of commercially available instrumentation.

In order to conduct sample analysis using the microarrays, polynucleotides are extracted from a biological sample. The biological samples may be obtained from any bodily fluid (blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. To produce probes, the polynucleotides extracted from the sample are used to produce nucleic acid sequences which are complementary to the nucleic acids on the microarray. If the microarray consists of cDNAs, antisense RNAs (aRNA) are appropriate probes. Therefore, in one aspect, mRNA is used to produce cDNA which, in turn and in the presence of fluorescent nucleotides, is used to produce fragment or oligonucleotide aRNA probes. These fluorescently labeled probes are incubated with the microarray so that the probe sequences hybridize to the cDNA oligonucleotides of the microarray. In another aspect, nucleic acid sequences used as probes can include polynucleotides, fragments, and complementary or antisense sequences produced using restriction enzymes, PCR technologies, and Oligolabeling or TransProbe kits (Pharmacia & Upjohn) well known in the area of hybridization technology.

Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with various degrees of less complementarity. After removal of nonhybridized probes, a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine the degree of

complementarity and the relative abundance of each oligonucleotide sequence on the microarray. A detection system may be used to measure the absence, presence, and amount of hybridization for all of the distinct sequences simultaneously. This data may be used for large scale correlation studies or for functional analysis of the sequences, mutations, variants, or polymorphisms among samples. (Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155.)

In another embodiment of the invention, nucleic acid sequences encoding TR2P may be used to generate hybridization probes useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, such as human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries, such as those reviewed in Price, C.M. (1993; Blood Rev. 7:127-134) and Trask, B.J. (1991; Trends Genet. 7:149-154).

Fluorescent in situ hybridization (FISH, as described, e.g., in Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, pp. 965-968, VCH Publishers New York, NY.) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding TR2P on a physical chromosomal map and a specific disorder, or predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by

genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, TR2P, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between TR2P and the agent being tested may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564 (Geysen, et al.). In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with TR2P, or fragments thereof, and washed. Bound TR2P is then detected by methods well known in the art. Purified TR2P can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding TR2P specifically compete with a test compound for binding TR2P. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with TR2P.

In additional embodiments, the nucleotide sequences which encode TR2P may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

EXAMPLES

I. cDNA Library Construction

SPLNNOT04

The SPLNNOT04 cDNA library was constructed from microscopically normal spleen tissue obtained from a 2-year-old Hispanic male who died of cerebral anoxia (specimen #RU95-09-0664; International Institute for the Advancement of Medicine, Exton, PA). The patient's serologies and past medical history were unremarkable.

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NJ) in guanidinium isothiocyanate solution. The lysate was centrifuged over a 5.7 M CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.7, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in RNAse-free water, and was treated with DNase at 37°C. RNA was extracted and precipitated as before. The mRNA was then isolated using the Qiagen Oligotex kit (QIAGEN, Inc., Chatsworth, CA) and used to construct the SPLNNOT04 cDNA library.

KIDNTUT13

The KIDNTUT13 cDNA library was constructed from cancerous left upper pole kidney tissue obtained from a 51-year-old Caucasian female during a nephroureterectomy. Pathology of the left kidney indicated a grade 3 renal cell carcinoma of clear cell type forming a hemorrhagic and cystic upper pole mass, adherent to the capsule. The renal vein was free of tumor. The non-neoplastic renal parenchyma and adrenal gland were unremarkable. The ureter margin was negative for tumor. The patient presented with backache. Patient history included depressive disorder, agoraphobia with panic, hypoglycemia, functional diarrhea, dysphagia, joint pain in multiple joints, uterine endometriosis, a normal delivery, and adult maltreatment syndrome. Previous surgeries included a total abdominal hysterectomy and an adenotonsillectomy. Patient medications included Triamterene, Propranolol Hydrochloride, Prilosec® (omeprazole; Astra/Merck Group, Wayne, PA), Vitamins E, C, and beta carotene, Questran® Light (Cholestyramine for Oral Suspension; Bristol-Myers Squibb Company, Princeton, NJ), Trazadone Hydrochloride, and protein supplements. Family history included benign hypertension,

malignant colon neoplasm, diabetes type II in grandparent; anxiety state and open chest coronary artery angioplasty in the father, and calculus of the kidney in the mother, father, and sibling.

The frozen tissue was homogenized and lysed in Trizol reagent (1 g tissue/10 ml Trizol; Cat. #10296-028; GIBCO-BRL, Gaithersburg, MD), a monoplastic solution of phenol and guanidine isothiocyanate, using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments). After a brief incubation on ice, chloroform was added (1:5 v/v) and the lysate was centrifuged. The upper chloroform layer was removed to a fresh tube and the RNA extracted with isopropanol, resuspended in DEPC-treated water, and treated with DNase for 25 min at 37°C. RNA was extracted and precipitated as before. The mRNA was then isolated using the Qiagen Oligotex kit (QIAGEN, Inc.) and used to construct the KIDNTUT13 cDNA library.

RNA from both libraries were handled according to the recommended protocols in the SuperScript plasmid system (Cat. #18248-013; GIBCO-BRL). cDNA synthesis was initiated with a NotI-oligo d(T) primer. Double-stranded cDNA was blunted, ligated to EcoRI adaptors, digested with NotI, fractionated on a Sepharose CL4B column (Cat. #275105-01, Pharmacia), and those cDNAs exceeding 400 bp were ligated into the NotI and EcoRI sites of the pINCY vector (Incyte). The plasmid pINCY for both libraries were subsequently transformed into DH5α™ competent cells (Cat. #18258-012, GIBCO-BRL).

II. Isolation and Sequencing of cDNA Clones

For both libraries, plasmid DNA was released from the cells and purified using the REAL Prep 96 plasmid kit (Catalog #26173, QIAGEN, Inc.). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, GIBCO-BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with

Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems; and the reading frame was determined.

III. Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of homology using BLAST (Basic Local Alignment Search Tool). (Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms such as the one described in Smith, T. et al. (1992; Protein Engineering 5:35-51), could have been used when dealing with primary sequence patterns and secondary structure gap penalties. The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-10} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid sequences from the same clones were then searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for homology.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a

membrane on which RNAs from a particular cell type or tissue have been bound. (Sambrook, supra, ch. 7) and Ausubel, F.M. et al. (supra, ch. 4 and 16).

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score, which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding TR2P occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V. Extension of TR2P Encoding Polynucleotides

The nucleic acid sequences of Incyte Clones 1533650, 2581223 were used to design oligonucleotide primers for extending partial nucleotide sequences to full length. For each nucleic acid sequence, one primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in

length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO/BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat steps 4 through 6 for an additional 15 cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat steps 8 through 10 for an additional 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQuick™ (QIAGEN Inc., Chatsworth, CA), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μl of ligation buffer, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent E. coli cells (in 40 μl of appropriate media) were transformed with 3 μl of ligation mixture and cultured in 80 μl of SOC medium. (Sambrook, supra, Appendix A, p. 2.). After incubation for one hour at 37° C, the E. coli mixture was plated on Luria

Bertani (LB) agar (Sambrook, supra, Appendix A, p. 1) containing 2x Carb. The following day, several colonies were randomly picked from each plate and cultured in 150 μl of liquid LB/2x Carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μl of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μl from each sample was transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequences of SEQ ID NO:2, SEQ ID NO:4, are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2, SEQ ID NO:4, are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences) and labeled by combining 50 pmol of each oligomer and 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex G-25 superfine resin column (Pharmacia &

Upjohn). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba 1, or Pvu II (DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

VII. Microarrays

To produce oligonucleotides for a microarray, one of the nucleotide sequences of the present invention is examined using a computer algorithm which starts at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary structure that would interfere with hybridization. The algorithm identifies approximately 20 sequence-specific oligonucleotides of 20 nucleotides in length (20-mers). A matched set of oligonucleotides are created in which one nucleotide in the center of each sequence is altered. This process is repeated for each gene in the microarray, and double sets of twenty 20-mers are synthesized and arranged on the surface of the silicon chip using a light-directed chemical process, such as that described in Chee (supra).

In the alternative, a chemical coupling procedure and an ink jet device are used to synthesize oligomers on the surface of a substrate. (See Baldeschweiler, supra.) In another alternative, a grid array analogous to a dot or slot blot is used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system or thermal, UV, mechanical, or chemical bonding procedures. A typical array may be produced by hand or using available materials and machines and contain grids of 8 dots, 24 dots, 96 dots, 384 dots, 1536 dots, or 6144 dots. After hybridization, the microarray is

washed to remove nonhybridized probes, and a scanner is used to determine the levels and patterns of fluorescence. The scanned image is examined to determine the degree of complementarity and the relative abundance/expression level of each oligonucleotide sequence in the microarray.

VIII. Complementary Polynucleotides

Sequences complementary to the TR2P-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring TR2P. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding sequence of TR2P. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the TR2P-encoding transcript.

IX. Expression of TR2P

Expression of TR2P is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector is also used to express TR2P in E. coli. This vector contains a promoter for ß-galactosidase upstream of the cloning site, followed by sequence containing the amino-terminal Met and the subsequent seven residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with isopropyl beta-D-thiogalactopyranoside (IPTG) using standard methods produces a fusion protein which consists of the first 8 residues of ß-galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of TR2P into bacterial growth media which can be used directly in the following assay for activity.

X. Demonstration of TR2P Activity

The human embryonic kidney 293 cell line is used for all transfection experiments. Transient transfections for luciferase reporter assays, Western blotting, and coimmunoprecipitation experiments are performed by the $CaPO_4$ method as described previously (Hsu, H. et al., (1995) Cell 81:495-504). Luciferase reporter assays are also performed as described previously (Hsu et al., supra). Twenty-four hours after transfection, approximately 2 x 10^6 293 cells per 100-mm plate are washed once in ice-cold phosphate-buffered saline (PBS), pH 7.4, containing 5 mM EDTA. Proteins are extracted from each plate in 0.5 ml of lysis buffer-250 (50 mM Tris, pH 7.4, 250 mM NaCl, 0.1% Nonidet P-40, 10% glycerol, 50 mM NaF, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A) for 30 min on ice. Cellular residue is removed by centrifugation. The supernatant is supplemented with 10 μM $ZnCl_2$, transferred to 10 μl of protein A-Sepharose (Pharmacia), blocked with 2% bovine serum albumin (Boehringer Mannheim) and prebound to anti-FLAG (1 μl) or anti-TR2P extracellular domain (1 μl), and incubated for 3 h at 4°C. The beads are washed five times in lysis buffer-250, and bound proteins are separated by 10% SDS-polyacrylamide gel electrophoresis and then blotted onto nitrocellulose (Schleicher & Schuell). For Western analysis, the blotted nitrocellulose membrane is blocked by incubation in 5% skim milk in PBS containing 0.05% Tween 20 (PBST) for 1 h at room temperature and incubated with anti-HA (2000-fold dilution with 1% gelatin in PBST), anti-FLAG (300-fold diluted), or anti-TR2P (700-fold diluted) for 1 h. The blots are washed three times in PBST and incubated in 3000-fold diluted protein A conjugated with horseradish peroxidase and then visualized with the ECL chemiluminescence detection system (Amersham Corp.). Protein concentrations are determined by the Bradford assay (Bio-Rad).

XI. Production of TR2P Specific Antibodies

TR2P substantially purified using PAGE electrophoresis (Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The TR2P amino acid sequence is analyzed using DNASTAR software (DNASTAR, Inc.) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate

epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel F.M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, ch. 11, John Wiley & Sons, New York, NY) and by others.

Typically, the oligopeptides are 15 residues in length, and are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), following the procedure described in Ausubel et al., supra. Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XII. Purification of Naturally Occurring TR2P Using Specific Antibodies

Naturally occurring or recombinant TR2P is substantially purified by immunoaffinity chromatography using antibodies specific for TR2P. An immunoaffinity column is constructed by covalently coupling TR2P antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing TR2P are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of TR2P (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/TR2P binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and TR2P is collected.

XIII. Identification of Molecules Which Interact with TR2P

TR2P or biologically active fragments thereof are labeled with ^{125}I Bolton-Hunter reagent. (Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled TR2P, washed, and any wells with labeled TR2P complex are assayed. Data obtained using different concentrations of TR2P are used to calculate values for the number, affinity, and association of TR2P with the candidate molecules.

Various modifications and variations of the described methods and systems of the

invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

What is claimed is:

1. A substantially purified human tumor necrosis factor-R2-like protein (TR2P) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, and a fragment of SEQ ID NO:3.

2. A substantially purified variant of ABBR having at least 90% amino acid identity to the amino acid sequence of claim 1.

3. An isolated and purified polynucleotide sequence encoding the TR2P of claim 1.

4. An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence of claim 3.

5. A composition comprising the polynucleotide sequence of claim 3.

6. An isolated and purified polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 3.

7. An isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence of claim 3.

8. An isolated and purified polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a fragment of SEQ ID NO:2, and a fragment of SEQ ID NO:4.

9. An isolated and purified polynucleotide variant having at least 90% polynucleotide identity to the polynucleotide sequence of claim 8.

10. An isolated and purified polynucleotide sequence which is complementary to the polynucleotide sequence of claim 8.

11. An expression vector containing at least a fragment of the polynucleotide sequence of claim 3.

12. A host cell containing the expression vector of claim 11.

13. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, a fragment of SEQ ID NO:1, or a fragment of SEQ ID NO:3, the method comprising the steps of:

a) culturing the host cell of claim 12 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

14. A pharmaceutical composition comprising the TR2P of claim 1 in conjunction with a suitable pharmaceutical carrier.

15. A purified antibody which specifically binds to the TR2P of claim 1.

16. A purified agonist of the TR2P of claim 1.

17. A purified antagonist of the TR2P of claim 1.

18. A method for treating or preventing an osteogenesis disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 14.

19. A method for treating or preventing a developmental disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 14.

20. A method for treating or preventing a reproductive disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 14.

21. A method for treating or preventing an immunological disorder, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

22. A method for treating or preventing a neoplastic disorder, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 17.

23. A method for detecting a polynucleotide encoding TR2P in a biological sample containing nucleic acids, the method comprising the steps of:

(a) hybridizing the polynucleotide of claim 7 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and

(b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding TR2P in the biological sample.

24. The method of claim 23 wherein the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

```
          9           18           27           36           45           54
  G CCC TCT CCC CGC CCA TAT ATG TAG GGC AGC AGC AGG ATG GGC TTC TGG ACT

         63           72           81           90           99          108
TGG GCG GCC CCT CCG CAG GCG GAC CGG GGG CAA AGG AGG TGG CAT GTC GGT CAG

        117          126          135          144          153          162
GCA CAG CAG GGT CCT GTG TCC GCG CTG AGC CGC GCT CTC CCT GCT CCA GCA AGG

        171          180          189          198          207          216
ACC ATG AGG GCG CTG GAG GGG CCA GGC CTG TCG CTG CTG TGC CTG GTG TTG GCG
    M   R   A   L   E   G   P   G   L   S   L   L   C   L   V   L   A

        225          234          243          252          261          270
CTG CCT GCC CTG CTG CCG GTG CCG GCT GTA CGC GGA GTG GCA GAA ACA CCC ACC
L   P   A   L   L   P   V   P   A   V   R   G   V   A   E   T   P   T

        279          288          297          306          315          324
TAC CCC TGG CGG GAC GCA GAG ACA GGG GAG CGG CTG GTG TGC GCC CAG TGC CCC
Y   P   W   R   D   A   E   T   G   E   R   L   V   C   A   Q   C   P

        333          342          351          360          369          378
CCA GGC ACC TTT GTG CAG CGG CCG TGC CGC CGA GAC AGC CCC ACG ACG TGT GGC
P   G   T   F   V   Q   R   P   C   R   R   D   S   P   T   T   C   G
```

FIGURE 1A

```
                387                 396                 405                 414                 423                 432
CCG TGT CCA CCG CGC CAC TAC ACG CAG TTC TGG AAC TAC CTA GAG CGC TGC CGC
P   C   P   P   R   H   Y   T   Q   F   W   N   Y   L   E   R   C   R

                441                 450                 459                 468                 477                 486
TAC TGC AAC GTC CTC TGC GGG GAG CGT GAG GAG GAG GCA CGG GCT TGC CAC GCC
Y   C   N   V   L   C   G   E   R   E   E   E   A   R   A   C   H   A

                495                 504                 513                 522                 531                 540
ACC CAC AAC CGT GCC TGC CGC TGC CGC ACC GGC TTC TTC GCG CAC GCT GGT TTC
T   H   N   R   A   C   R   C   R   T   G   F   F   A   H   A   G   F

                549                 558                 567                 576                 585                 594
TGC TTG GAG CAC GCA TCG TGT CCA CCT GGT GCC GGC GTG ATT GCC CCG GGC ACC
C   L   E   H   A   S   C   P   P   G   A   G   V   I   A   P   G   T

                603                 612                 621                 630                 639                 648
CCC AGC CAG AAC ACG CAG TGC CAG CCG TGC CCC CCA GGC ACC TTC TCA GCC AGC
P   S   Q   N   T   Q   C   Q   P   C   P   P   G   T   F   S   A   S

                657                 666                 675                 684                 693                 702
AGT TCC AGC TCA GAG CAG TGC CAG CCC CAC CGC AAC TGC ACG GCC CTG GGC CTG
S   S   S   S   E   Q   C   Q   P   H   R   N   C   T   A   L   G   L

                711                 720                 729                 738                 747                 756
GCC CTC AAT GTG CCA GGC TCT TCC TCC CAT GAC ACC CTG TGC ACC AGC TGC ACT
A   L   N   V   P   G   S   S   S   H   D   T   L   C   T   S   C   T
```

FIGURE 1B

```
        765         774         783         792         801         810
GGC TTC CCC CTC AGC ACC AGG GTA CCA GGA GCT GAG GAG TGT GAG CGT GCC GTC
G   F   P   L   S   T   R   V   P   G   A   E   E   C   E   R   A   V

        819         828         837         846         855         864
ATC GAC TTT GTG GCT TTC CAG GAC ATC TCC ATC AAG AGG CTG CAG CGG CTG CTG
I   D   F   V   A   F   Q   D   I   S   I   K   R   L   Q   R   L   L

        873         882         891         900
CAG GCC CTC GAG GCC CCG GAG GAC TGG GGT CCG ACA C
Q   A   L   E   A   P   E   D   W   G   P   T
```

FIGURE 1C

```
          9          18          27          36          45          54
TTG TTT TCA TAT ATT TGT GTT AAT TTC CTT TAT ATA GGT ACA ATG TTG TTT GTG

         63          72          81          90          99         108
TTC GTT TCT AGA TCT AGT GGA TCC TCC GTT CTT CAG TAA TTC GGC ATT TGC CGC

        117         126         135         144         153         162
CAT GTT GAC CGT GAC CAC CGG CCA GGT GCT AAC CTG TGA CAA GTG TCC AGC AGG

        171         180         189         198         207         216
AAC CTA TGT CTC TGA GCA TTG TAC CAA CAC AAG CCT GCG CGT CTG CAG CAG TTG

        225         234         243         252         261         270
CCC TGT GGG GAC CCT TAC CAG GCA TGG GGN GGG CAT AGA GTT TTT TNC ATG ATT

        279         288         297         306         315         324
TTA GTC AGC CAT GCC CAT GGC CAA TGA ATT GAG AAA TTA CCT TGT GCT GCC TTG
                                    I   E   K   L   P   C   A   A   L

        333         342         351         360         369         378
ACT GAC CGA GAA TGC ACT TGC CCA CCT GGC ATG TTC CAG TCT AAC GCT ACC TGT
T   D   R   E   C   T   C   P   P   G   M   F   Q   S   N   A   T   C

        387         396         405         414         423         432
GCC CCC CAT ACG GTG TGT CCT GTG GGT TGG GGT GTG CGG AAG AAA GGG ACA GAG
A   P   H   T   V   C   P   V   G   W   G   V   R   K   K   G   T   E
```

FIGURE 2A

```
                441                 450                 459                 468                 477                 486
ACT GAG GAT GTG CGG TGT AAG CAG TGT GCT CGG GGT ACC TTC TCA GAT GTG CCT
T   E   D   V   R   C   K   Q   C   A   R   G   T   F   S   D   V   P

                495                 504                 513                 522                 531                 540
TCT AGT GTG ATG AAA TGC AAA GCA TAC ACA GAC TGT CTG AGT CAG AAC CTG GTG
S   S   V   M   K   C   K   A   Y   T   D   C   L   S   Q   N   L   V

                549                 558                 567                 576                 585                 594
GTG ATC AAG CCG GGG ACC AAG GAG ACA GAC AAC GTC TGT GGC ACA CTC CCG TCC
V   I   K   P   G   T   K   E   T   D   N   V   C   G   T   L   P   S

                603                 612                 621                 630                 639                 648
TTC TCC AGC TCC ACC TCA CCT TCC CCT GGC ACA GCC ATC TTT CCA CGC CCT GAG
F   S   S   S   T   S   P   S   P   G   T   A   I   F   P   R   P   E

                657                 666                 675                 684                 693                 702
CAC ATG GAA ACC CAT GAA GTC CCT TCC TCC ACT TAT GTT CCC AAA GGC ATG AAC
H   M   E   T   H   E   V   P   S   S   T   Y   V   P   K   G   M   N

                711                 720                 729                 738                 747                 756
TCA ACA GAA TCC AAC TCT TCT GCC TCT GTT AGA CCA AAG GTA CTG AGT AGC ATC
S   T   E   S   N   S   S   A   S   V   R   P   K   V   L   S   S   I

                765                 774                 783                 792                 801                 810
CAG GAA GGG ACA GTC CCT GAC AAC ACA AGC TCA GCA AGG GGG AAG GAA GAC GTG
Q   E   G   T   V   P   D   N   T   S   S   A   R   G   K   E   D   V
```

FIGURE 2B

```
          819         828         837         846         855         864
AAC AAG ACC CTC CCA AAC CTT CAG GTA GTC AAC CAC CAG CAA GGC CCC CAC CAC
N   K   T   L   P   N   L   Q   V   V   N   H   Q   Q   G   P   H   H

          873         882         891         900         909         918
AGA CAC ATC CTG AAG CTG CTG CCG TCC ATG GAG GCC ACT GGG GGC GAG AAG TCC
R   H   I   L   K   L   L   P   S   M   E   A   T   G   G   E   K   S

          927         936         945         954         963         972
AGC ACG CCC ATC AAG GGC CCC AAG AGG GGA CAT CCT AGA CAG AAC CTA CAC AAG
S   T   P   I   K   G   P   K   R   G   H   P   R   Q   N   L   H   K

          981         990         999        1008        1017        1026
CAT TTT GAC ATC AAT GAG CAT TTG CCC TGG ATG ATT GTG CTT TTC CTG CTG CTG
H   F   D   I   N   E   H   L   P   W   M   I   V   L   F   L   L   L

         1035        1044        1053        1062        1071        1080
GTG CTT GTG GTG ATT GTG GTG TGC AGT ATC CGG AAA AGC TCG AGG ACT CTG AAA
V   L   V   V   I   V   V   C   S   I   R   K   S   S   R   T   L   K

         1089        1098        1107        1116        1125        1134
AAG GGG CCC CGG CAG GAT CCC AGT GCC ATT GTG GAA AAG GCA GGG CTG AAG AAA
K   G   P   R   Q   D   P   S   A   I   V   E   K   A   G   L   K   K

         1143        1152        1161        1170        1179        1188
TCC ATG ACT CCA ACC CAG AAC CGG GAG AAA TGG ATC TAC TAC TGC AAT GGC CAT
S   M   T   P   T   Q   N   R   E   K   W   I   Y   Y   C   N   G   H
```

FIGURE 2C

```
        1197        1206        1215        1224        1233        1242
GGT ATC GAT ATC CTG AAG CTT GTA GCA GCC CAA GTG GGA AGC CAG TGG AAA GAT
G   I   D   I   L   K   L   V   A   A   Q   V   G   S   Q   W   K   D

        1251        1260        1269        1278        1287        1296
ATC TAT CAG TTT CTT TGC AAT GCC AGT GAG AGG GAG GTT GCT GCT TTC TCC AAT
I   Y   Q   F   L   C   N   A   S   E   R   E   V   A   A   F   S   N

        1305        1314        1323        1332        1341        1350
GGG TAC ACA GCC GAC CAC GAG CGG GCC TAC GCA GCT CTG CAG CAC TGG ACC ATC
G   Y   T   A   D   H   E   R   A   Y   A   A   L   Q   H   W   T   I

        1359        1368        1377        1386        1395        1404
CGG GGC CCC GAG GCC AGC CTC GCC CAG CTA ATT AGC GCC CTG CGC CAG CAC CGG
R   G   P   E   A   S   L   A   Q   L   I   S   A   L   R   Q   H   R

        1413        1422        1431        1440        1449        1458
AGA AAC GAT GTT GTG GAG AAG ATT CGT GGG CTG ATG GAA GAC ACC ACC CAG CTG
R   N   D   V   V   E   K   I   R   G   L   M   E   D   T   T   Q   L

        1467        1476
GAA ACT GAC AAA CTA GCT
E   T   D   K   L   A
```

FIGURE 2D

```
1   MRALE------GPGLSLLCLV 1533650
1   MN----------------KLLLCCA GI 2072185
1   MAPVAVWAALAVGLELWAAA GI 1469541

16  LALPALLPVPAVRGVAET-- 1533650
9   LVF---LDISIKWTTQETFP GI 2072185
21  HALPAQVAFTPY--APEPGS GI 1469541

34  PTYPWRDAETGERLVCAQCP 1533650
26  PKYLHYDEETSHQLLCDKCP GI 2072185
39  TCRLREYYDQTAQMCCSKCS GI 1469541

54  PGTFVQRPCRRDSPTTCGPC 1533650
46  PGTYLKQHCTAKWKTVCAPC GI 2072185
59  PGQHAKVFCTKTSDTVCDSC GI 1469541

74  PPRHYTQFWNYLERCRYCNV 1533650
66  PDHYYTDSWHTSDECLYCSP GI 2072185
79  EDSTYTQLWNWVPECLSCGS GI 1469541

94  LCGEREEEARACHATHNRAC 1533650
86  VCKELQYVKQECNRTHNRVC GI 2072185
99  RCSSDQVETQACTREQNRIC GI 1469541

114 RCRTGFFAHAG------FCL 1533650
106 ECKEGRYLEIE------FCL GI 2072185
119 TCRPGWYCALSKQEGCRLCA GI 1469541

128 EHASCPPGAGVIAPGTPSQN 1533650
120 KHRSCPPGFGVVQAGTPERN GI 2072185
139 PLRKCRPGFGVARPGTETSD GI 1469541

148 TQCQPCPPGTFSASSSSSEQ 1533650
140 TVCKRCPDGFFSNETSSKAP GI 2072185
159 VVCKPCAPGTFSNTTSSTDI GI 1469541
```

FIGURE 3A

```
168 C Q P H R N C T A L G L A L N V P G S S 1533650
160 C R K H T N C S V F G L L L T Q K G N A GI 2072185
179 C R P H Q I C N V V A I - - - - P G N A GI 1469541

188 S H D T L C T S - - - - - - - - - - - - - 1533650
180 T H D N I C S G N S E S T Q K C G I D V GI 2072185
195 S M D A V C T S T S P T R S M A P G A V GI 1469541

196 - - C T G - - - - F - - - - - P L S T R - 1533650
200 T L C E E A F F R F - - - - A V P T K F GI 2072185
215 H L P Q P V S T R S Q H T Q P T P E P S GI 1469541

205 - - - - - - - - - - - - V P G A - - - - - 1533650
216 T P N W L S V L V D N L P G T K V N A E GI 2072185
235 T A P S T S F L L P M G P S P P A E G S GI 1469541

209 - - - - - - - - - - - - - - - - - - - - 1533650
236 S V E - - - - - - - - - - - - - - - - - GI 2072185
255 T G D F A L P V G L I V G V T A L G L L GI 1469541

209 - - - - - - - - - - - - - - - - - - - - 1533650
239 - - - - - - - - - - - - R I K R Q H S S Q GI 2072185
275 I I G V V N C V I M T Q V K K K P L C L GI 1469541

209 - - - - - - - - - - - - - - - - - - - - 1533650
248 E Q T F Q L L K L W K H Q N K A Q D I V GI 2072185
295 Q R E A K V P H L P A D K A R G T Q G P GI 1469541

209 - - - - - - - - - - E E C E R A V I D F 1533650
268 K K - - - I I Q D I D L C E N S V Q R H GI 2072185
315 E Q Q H L L I T A P S S S S S S L E S S GI 1469541

219 V - A F Q D I S I K R L Q R L L Q A L E 1533650
285 I - G H A N L T F E Q L R S L M E S L P GI 2072185
335 A S A L D R R A P T R N Q P Q A P G V E GI 1469541
```

FIGURE 3B

```
238 A----------------PED 1533650
304 GKKVGAEDIEKTIKACKPSD GI 2072185
355 ASGAGEARASTGSSDSSPGG GI 1469541

242 ----------W---------- 1533650
324 Q--ILKLLSLWRI-KNGDQD GI 2072185
375 HGTQVNVTCIVNVCSSSDHS GI 1469541

243 -------------------- 1533650
341 TLKGLMHALKHSKTYHFPKT GI 2072185
395 SQCSSQASSTMGDTDSSPSE GI 1469541

243 -------------------- 1533650
361 VTQSLKKTIRFLHSFTMYKL GI 2072185
415 SPK--DEQVPFSKEECAFRS GI 1469541

243 -------------------- 1533650
381 YQKLFLEMIGNQ--------- GI 2072185
433 QLETPETLLGSTEEKPLPLG GI 1469541

243 ------GPT 1533650
393 VQSVKISCL GI 2072185
453 VPDAGMKPS GI 1469541
```

FIGURE 3C

```
1   IE--KLPCAALTDRECTCPP 2581223
1   VE--TQACTREQNRICTCRP GI 1469541
1   LQYVKQECNRTHNRVCECKE GI 2072185

19  GMFQSNAT------CAPHTV 2581223
19  GWYCALSKQEGCRLCAPLRK GI 1469541
21  GRYLEIE------FCLKHRS GI 2072185

33  CPVGWGVRKKGTETEDVRCK 2581223
39  CRPGFGVARPGTETSDVVCK GI 1469541
35  CPPGFGVVQAGTPERNTVCK GI 2072185

53  QCARGTFSDVPSSVMKCKAY 2581223
59  PCAPGTFSNTTSSTDICRPH GI 1469541
55  RCPDGFFSNETSSKAPCRKH GI 2072185

73  TDCLSQNLVVIKPGTKETDN 2581223
79  QICNVVAI----PGNASMDA GI 1469541
75  TNCSVFGLLLTQKGNATHDN GI 2072185

93  VCGTLPSFSSSTSPSPG--T 2581223
95  VCTSTSPTRSMAPGAVHLPQ GI 1469541
95  ICSGNSESTQKCGIDVTLCE GI 2072185

111 AIFPRPEHMETHEVPSST-- 2581223
115 PVSTRSQHTQPTPEPSTAPS GI 1469541
115 ---------------------- GI 2072185

129 ---YVPKGMNSTESNSSA-- 2581223
135 TSFLLPMGPSPPAEGSTGDF GI 1469541
115 EAFF----------------RF GI 2072185

144 SVRPK----------VLSSI 2581223
155 ALPVGLIVGVTALGLLIIGV GI 1469541
121 AVPTKFTPNW--LSVLVDNL GI 2072185
```

FIGURE 4A

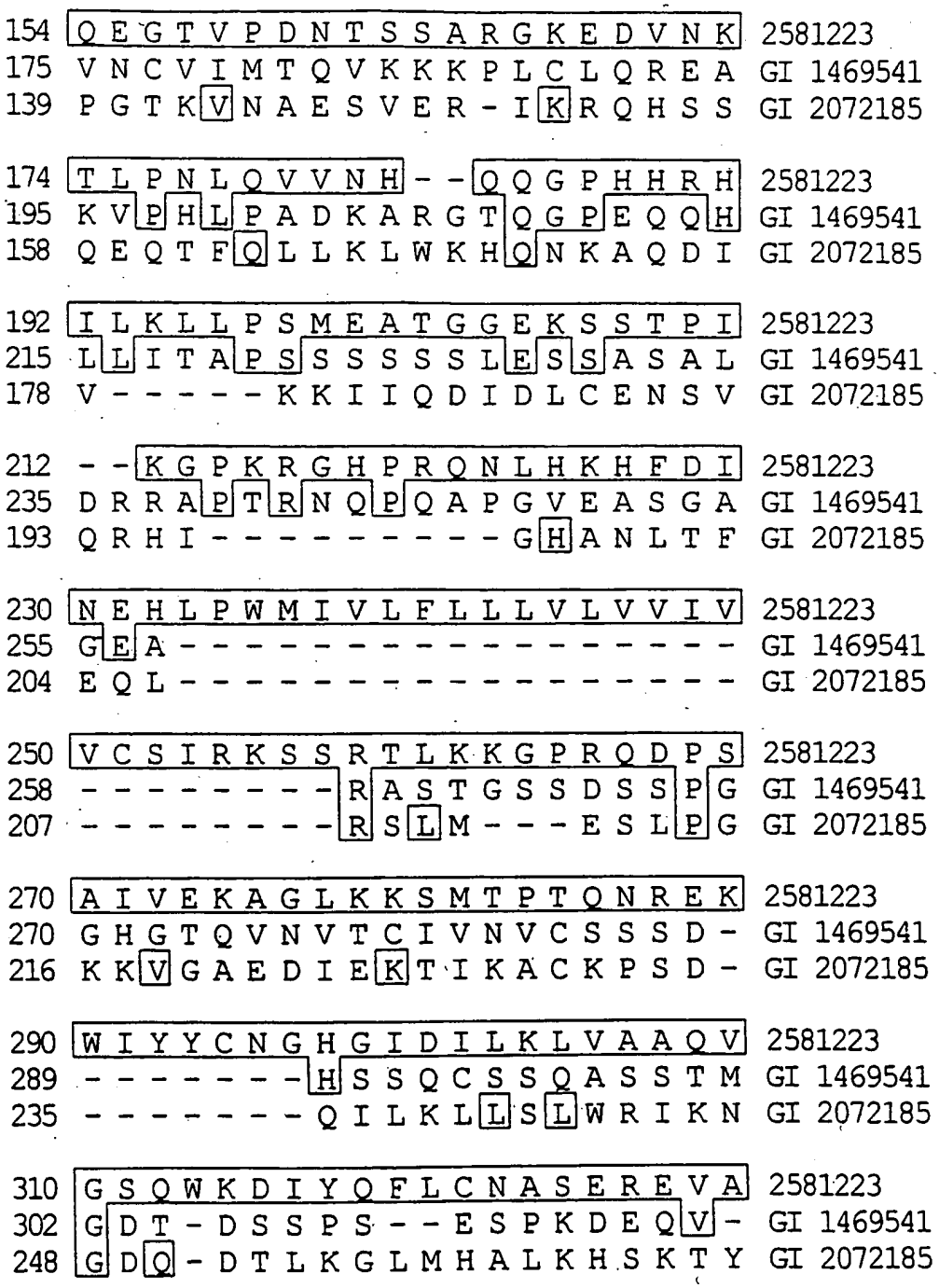
154 Q E G T V P D N T S S A R G K E D V N K 2581223
175 V N C V I M T Q V K K K P L C L Q R E A GI 1469541
139 P G T K V N A E S V E R - I K R Q H S S GI 2072185
174 T L P N L Q V V N H - - Q Q G P H H R H 2581223
195 K V P H L P A D K A R G T Q G P E Q Q H GI 1469541
158 Q E Q T F Q L L K L W K H Q N K A Q D I GI 2072185
192 I L K L L P S M E A T G G E K S S T P I 2581223
215 L L I T A P S S S S S S L E S S A S A L GI 1469541
178 V - - - - - K K I I Q D I D L C E N S V GI 2072185
212 - - K G P K R G H P R Q N L H K H F D I 2581223
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FIGURE 4B

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FIGURE 4C

SEQUENCE LISTING

<110> INCYTE PHARMACEUTICALS, INC.
BANDMAN, Olga
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AU-YOUNG, Janice
TANG, Y. Tom
KASER, Matthew R.

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C12N 15/12, 5/10, C07K 14/705, 16/28, C12Q 1/68, A61K 38/17

A3

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(30) Priority Data:
08/991,945 16 December 1997 (16.12.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/991,945 (CIP)
Filed on 16 December 1997 (16.12.97)

(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BANDMAN, Olga [US/US]; 366 Anna Avenue, Mountain View, CA 94043 (US). HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Berkeley, CA 94702 (US). TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). KASER, Matthew, R. [GB/US]; 4793 Ewing Road, Castro Valley, CA 94546-1017 (US).

(74) Agents: BILLINGS, Lucy, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(57) Abstract

The invention provides human tumor necrosis factor-R2-like proteins (TR2P) and polynucleotides which identify and encode TR2P. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of TR2P.

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INTERNATIONAL SEARCH REPORT

Inte 'lonal Application No
PCT/US 98/25649

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C07K14/705 C07K16/28 C12Q1/68
A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 23614 A (AMGEN INC ;LACEY DAVID L (US); BOYLE WILLIAM J (US); CALZONE FRANK) 3 July 1997 (1997-07-03) abstract claims 1-60 figure 2 ---	1-24
P,X	EP 0 861 850 A (SMITHKLINE BEECHAM CORP) 2 September 1998 (1998-09-02) abstract claims 1-25 figure 1 --- -/--	1-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 May 1999	26.08.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

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INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/US 98/25649

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 30694 A (HUMAN GENOME SCIENCES INC ;FENG PING (US); NI JIAN (US); EBNER REI) 16 July 1998 (1998-07-16) abstract claims 1-23 figures 1-3 ---	1-24
E	WO 99 04001 A (ZYMOGENETICS INC) 28 January 1999 (1999-01-28) abstract claims 1-24 figure 1 ---	1-24
E	WO 99 07738 A (MASIAKOWSKI PIOTR J ;PROCTER & GAMBLE COMPAGNY (US); MORRIS JODI () 18 February 1999 (1999-02-18) abstract claims 1-18 see seq. IDs 1, 2 ---	1-24
E	WO 99 14330 A (GENENTECH INC) 25 March 1999 (1999-03-25) abstract claims 1-66 figures 5,6 -----	1-24

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 98/25649

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: -
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 18-22 are directed to methods of treatment. The search has been limited to the alleged effects of the compounds/composition.

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

(1-24) - partial

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1-24) - partial

A human tumor necrosis factor-R2-like protein comprising amino acid of sequence ID 1 or a fragment thereof.

Corresponding homologs, nucleic acids (seq. ID 3), vectors, host cells, compositions, pharmaceutical formulations, therapeutic and diagnotic uses, agonists and antagonists.

2. Claims: (1-24) - partial

Idem as subject matter 1, but relating to seq. IDs 2 and 4.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The agonists and antagonists of claims 16,17,21,22 are not sufficiently described to allow for a meanigful and complete search.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/25649

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